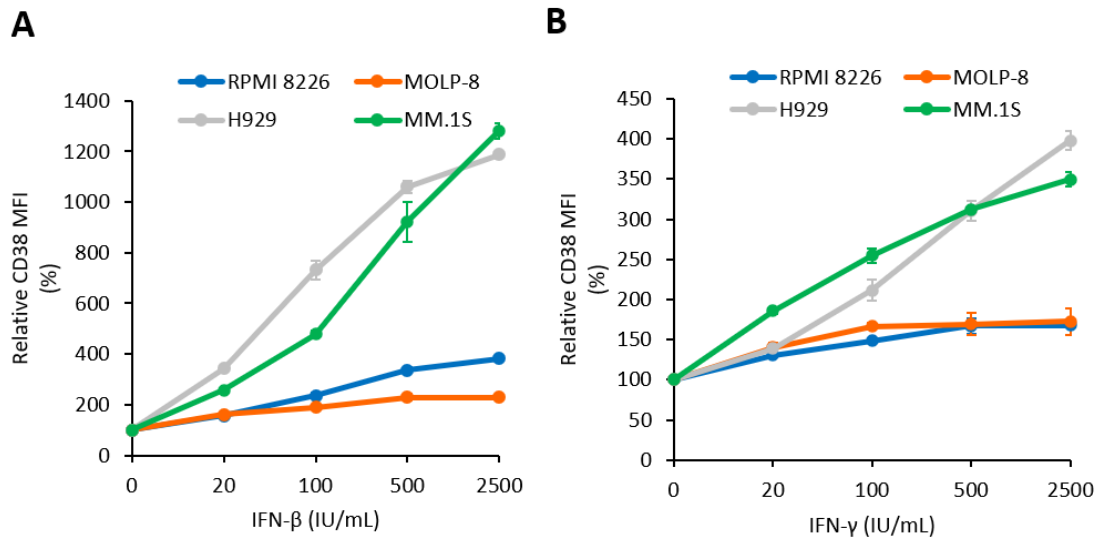


Supplemental Figures

Supplemental Figure 1



Supplemental Figure 1. IFN- β and IFN- γ increase CD38 expression on MM cells. (A, B)

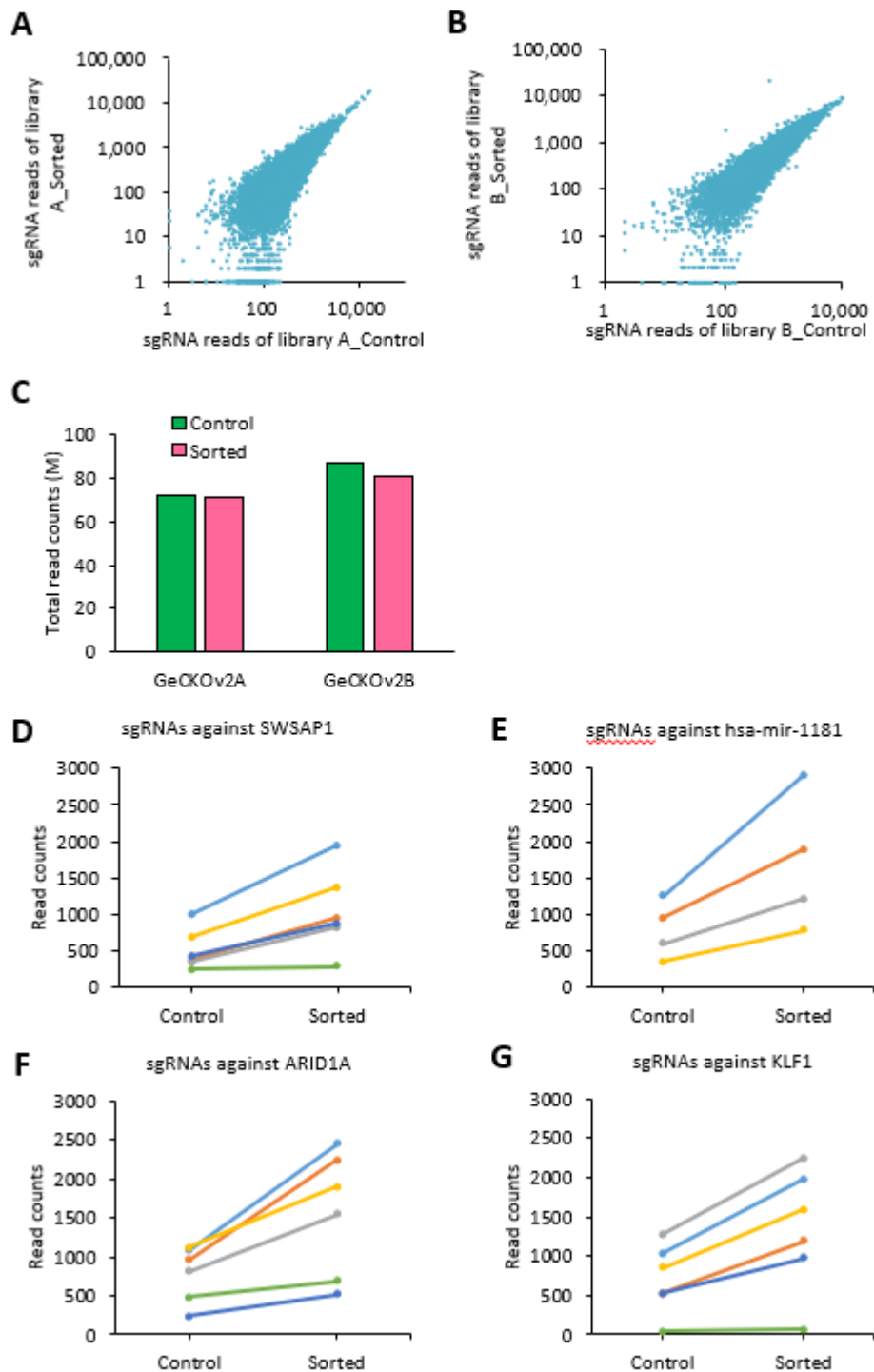
RPMI 8226, MOLP-8, H929, and MM.1S cells were cultured with IFN- β (0 to 2500 IU/mL)

(A) or IFN- γ (0 to 2500 IU/mL) (B) for 72h. After incubation, CD38 expression was measured

by flow cytometry, and relative CD38 MFI was calculated in comparison with MFI of Control.

Data are shown as mean \pm standard error of the mean.

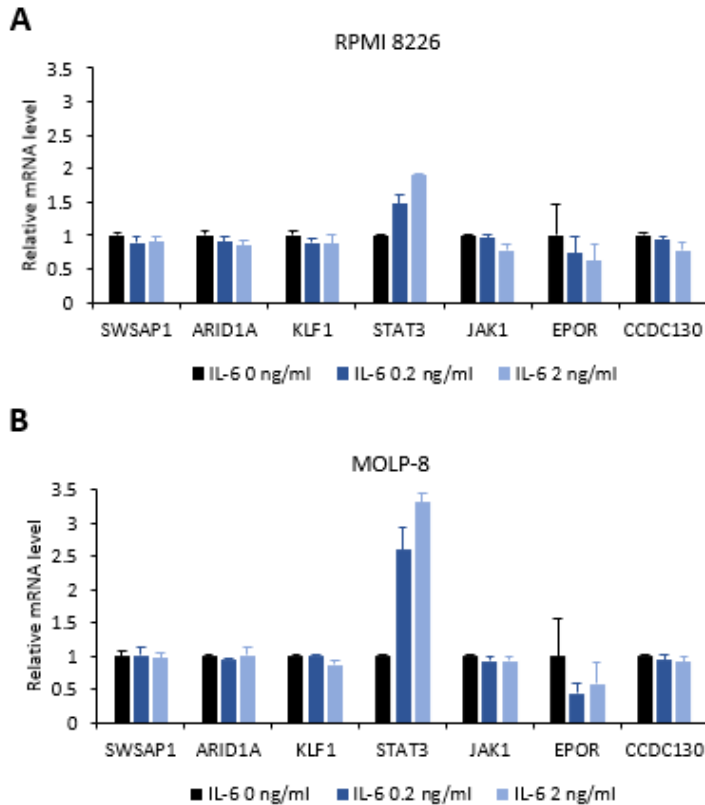
Supplemental Figure 2



Supplemental Figure 2. Genome-scale CRISPR-Cas9 knockout screening in RPMI

8226 cells. (A, B) Scatter plot of sgRNA representation between control cells and sorted cells in the GeCKO library A (A) and library B (B). (C) Comparison of total sgRNA reads between control and sorted cells in two libraries. (D-G) Read counts in top 5 genes (*SWSAP1* (D), *hsa-mir-1181* (E), *ARID1A* (F), and *KLF1* (G)) enriched in sorted cells compared to control cells. *SWSAP1*, *SWIM-type zinc finger 7 associated protein 1*; *ARID1A*, *AT-rich interaction domain 1A*; *KLF1*, *Kruppel like factor 1*.

Supplemental Figure 3



Supplemental Figure 3. Validation of top positive genes identified by genome-scale

CRISPR-Cas9 knockout screening. (A, B) qRT-PCR analysis of some genes in high

positive rank listed in Figure 3B was carried out in RPMI 8226 (A) and MOLP-8 cells (B)

treated with control culture medium or IL-6 (0.2 or 2 ng/ml) for 6 h. The values were

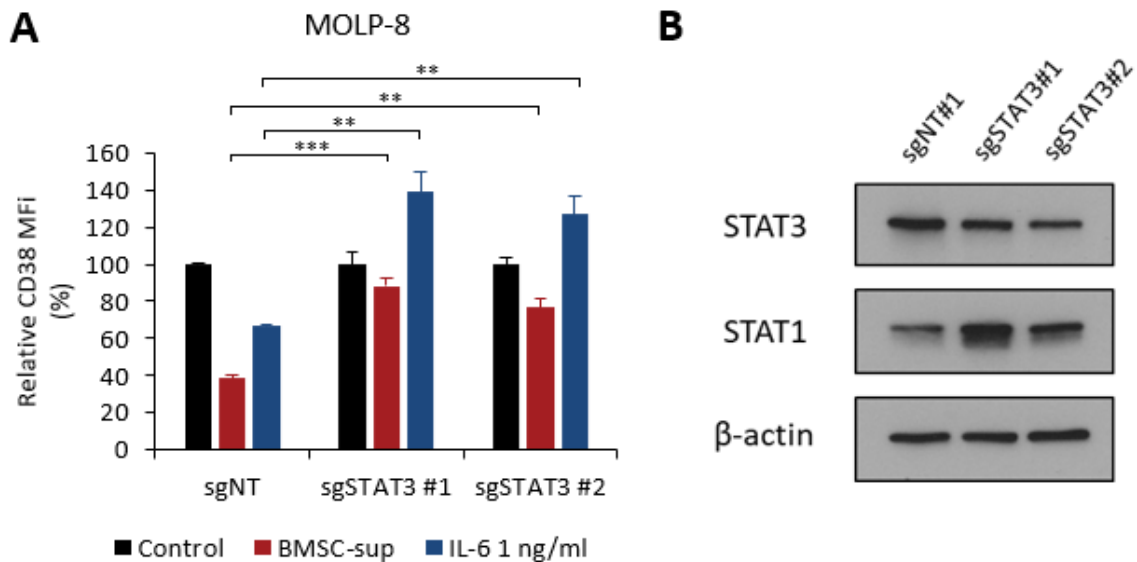
normalized to invariant control *GAPDH* expression. Data are shown as mean \pm standard

error of the mean. Specific primers for each gene are listed in Supplemental Table 2.

SWSAP1, SWIM-type zinc finger 7 associated protein 1; *ARID1A*, AT-rich interaction domain

1A; *KLF1*, Kruppel like factor 1; *JAK1*, Janus kinase 1; *EPOR*, Erythropoietin receptor, *CCDC130*, Coiled-coil domain containing 130; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

Supplemental Figure 4



Supplemental Figure 4. *STAT3* knockout inhibits CD38 downregulation induced by BMSC-sup or IL-6. (A, B) MOLP-8 cells were infected with sgNT (non-target control), sgSTAT3 #1 or #2 expressing lentiviral vector, and cultured with control culture medium (Control), BMSC-sup or IL-6 (1 ng/ml) for 72 h. (A) CD38 expression was assessed by flow cytometry. Relative CD38 MFI was calculated in comparison with MFI of Control. (B) STAT3 downregulation was confirmed by immunoblotting.

Supplemental Tables

Supplemental Table 1. Antibodies used in this study

Antibodies	Sources	Catalog #	Applications
STAT1	Cell signaling technology	9176	WB
STAT3	Cell signaling technology	9139	WB
phospho-STAT1 (Tyr701)	Cell signaling technology	7649	WB
phospho-STAT3 (Tyr705)	Cell signaling technology	9145	WB
phospho-STAT3 (Ser727)	Cell signaling technology	9134	WB
FLAG-Tag	Cell signaling technology	14793	WB
β -actin-HRP	Cell signaling technology	12262	WB
rabbit IgG-HRP	Cell signaling technology	7074	WB (2nd Ab)
mouse IgG-HRP	Cell signaling technology	7076	WB (2nd Ab)
CD38 PE/Cy7	BioLegend	356608	FC
IgG1, κ PE/Cy7	BioLegend	400126	FC
CD138 BV421	BioLegend	356516	FC
IgG1, κ BV421	BioLegend	400158	FC
7-AAD	BD Pharmingen	559925	FC

WB, western blot; HRP, horseradish peroxidase; Ab, antibody; FC, flow cytometry; PE/Cy7, phycoerythrin-cyanin 7; BV421, Brilliant Violet 421; 7-AAD, 7-amino-actinomycin D.

Supplemental Table 2. Primers for quantitative real-time PCR

Target genes	Directions	Sequences (5' to 3')
<i>CD38</i>	Forward	CAGCAACAACCCTGTTTCAGT
<i>CD38</i>	Reverse	CCATTGAGCATCACATGGAC
<i>STAT3</i>	Forward	CCTCTGCCGGAGAAACAGT
<i>STAT3</i>	Reverse	CATTGGGAAGCTGTCACTGTAG
<i>SWSAP1</i>	Forward	GGAACGACTCTAGACCCAATGC
<i>SWSAP1</i>	Reverse	CCTCATGGGCAGAGCACAGGA
<i>ARID1A</i>	Forward	AAGCCACCAACTCCAGCATCCA
<i>ARID1A</i>	Reverse	CGCTTCTGGAATGTGGAGTCAC
<i>KLF1</i>	Forward	TTGCGGCAAGAGCTACACCAAG
<i>KLF1</i>	Reverse	GTAGTGGCGGGTCAGCTCGTC
<i>JAK1</i>	Forward	GAGACAGGTCTCCCACAAACAC
<i>JAK1</i>	Reverse	GTGGTAAGGACATCGCTTTTCCG
<i>EPOR</i>	Forward	TTCTGTGCTTCACCGAGCGGTT
<i>EPOR</i>	Reverse	CGACACAGCTTCCATGGCTCAT
<i>CCDC130</i>	Forward	CTCTCAACCGATACCACAACAGC
<i>CCDC130</i>	Reverse	CATGCCGATGTGGTTCTTG CAG
<i>GAPDH</i>	Forward	CCTGCACCACCAACTGCTT
<i>GAPDH</i>	Reverse	CCATCACGCCACAGTTTCC

SWSAP1, SWIM-type zinc finger 7 associated protein 1; *ARID1A*, AT-rich interaction domain 1A; *KLF1*, Kruppel like factor 1; *JAK1*, Janus kinase 1; *EPOR*, Erythropoietin receptor; *CCDC130*, Coiled-coil domain containing 130; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

Supplemental Table 3. Plasmid vectors used in this study

Plasmids	Sources	Catalog #
lentiCRISPR v2	Addgene	52961
pCMV-dR8.2 dvpr	Addgene	8455
pCMV-VSV-G	Addgene	8454
EF.STAT3DN.Ubc.GFP	Addgene	24984
EF.STAT3C.Ubc.GFP	Addgene	24983
MSCV IRES Luciferase	Addgene	18760

Supplemental Table 4. sgRNAs used in this study

Target genes	Vectors	Clone IDs	Target sequences
<i>STAT3</i>	sgRNA	HGLibA_47386	AGATTGCCCGGATTGTGGCC
<i>STAT3</i>	sgRNA	HGLibB_47330	ACTGCTGGTCAATCTCTCCC
<i>Non-target</i>	sgRNA	HGLibA_64388	CCATATCGGGGCGAGACATG

sgRNA, single guide RNA.

Supplemental Table 5. Relative CD38 mean fluorescence intensities of cytokine profiling in 4 MM cell lines

	Concentration	RPMI8226	MOLP8	MM.1S	H929
IL-6	0.1 ng/ml	75.5	74.8	96.8	90.0
	1 ng/ml	57.8	65.3	83.1	78.2
	10 ng/ml	58.5	62.2	76.6	78.5
MIP-1 α	1 ng/ml	107.5	101.5	99.5	106.5
	10 ng/ml	107.6	99.8	99.5	98.3
	100 ng/ml	106.8	94.7	100.8	97.8
SDF-1 α	1 ng/ml	110.1	93.5	102.0	94.6
	10 ng/ml	106.6	98.1	103.9	90.6
	100 ng/ml	106.1	104.0	104.0	91.0
IL-1 β	1 ng/ml	102.3	102.9	106.3	90.5
	5 ng/ml	100.1	110.7	103.6	93.7
	25 ng/ml	102.0	113.8	105.3	96.6
IL-8	5 ng/ml	99.6	101.4	96.0	103.2
	25 ng/ml	99.3	101.1	100.9	100.5
	100 ng/ml	99.6	108.3	99.8	110.1
IGF-1	25 ng/ml	98.8	87.2	98.0	104.6
	100 ng/ml	87.7	81.9	100.8	93.1
	250 ng/ml	83.7	83.0	105.5	98.7
TGF- β	0.2 ng/ml	125.1	89.1	97.1	104.9
	1 ng/ml	137.9	87.1	98.7	100.9
	5 ng/ml	134.5	87.8	100.3	93.0
OSM	1 ng/ml	96.9	98.0	96.9	95.2
	10 ng/ml	96.5	98.7	98.6	92.5
	100 ng/ml	97.4	99.6	96.6	94.2
LIF	1 ng/ml	96.2	96.7	98.4	98.5
	10 ng/ml	96.1	99.1	98.3	98.5
	100 ng/ml	97.5	99.5	98.0	101.5
IL-10	1 ng/ml	76.8	97.2	99.2	101.1

	10 ng/ml	65.7	95.1	98.3	98.2
	100 ng/ml	64.7	99.6	101.0	96.0
IL-2	1 ng/ml	105.0	108.4	102.2	99.3
	10 ng/ml	98.4	105.5	101.1	97.7
	100 ng/ml	99.8	102.8	99.2	92.6
IFN- β	20 IU/ml	156.3	160.1	256.9	342.2
	100 IU/ml	235.9	190.2	478.8	731.9
	500 IU/ml	336.9	229.8	923.1	1060.7
IFN- γ	20 IU/ml	130.5	140.5	185.7	138.8
	100 IU/ml	148.6	166.6	254.7	211.6
	500 IU/ml	167.0	169.5	312.0	311.0

IL-6, interleukin-6; MIP-1 α , macrophage inflammatory protein-1 α ; SDF-1 α , stromal cell-derived factor-1 α ; IL-1 β , interleukin-1 β ; IL-8, interleukin-8; IGF-1, insulin-like growth factor 1; TGF- β , transforming growth factor- β ; OSM, oncostatin-M; LIF, leukemia inhibitory factor; IL-10, interleukin-10; IL-2, interleukin-2; IFN- β , interferon- β ; IFN- γ , interferon- γ .

Supplemental Table 6. p-value of student t-test in cytokine profiling in 4 MM cell lines

	Concentration	RPMI8226	MOLP8	MM.1S	H929
IL-6	0.1 ng/ml	0.0002	0.0080	0.0007	0.0049
	1 ng/ml	0.0001	0.0000	0.0003	0.0013
	10 ng/ml	0.0000	0.0000	0.0002	0.0005
MIP-1 α	1 ng/ml	0.0105	0.5623	0.8810	0.0377
	10 ng/ml	0.0098	0.9707	0.8883	0.6629
	100 ng/ml	0.0658	0.1158	0.8087	0.1227
SDF-1 α	1 ng/ml	0.0355	0.0673	0.5484	0.0062
	10 ng/ml	0.2317	0.7216	0.2954	0.0072
	100 ng/ml	0.0182	0.1936	0.2848	0.0007
IL-1 β	1 ng/ml	0.7635	0.4836	0.1463	0.0185
	5 ng/ml	0.9712	0.0812	0.3251	0.0723
	25 ng/ml	0.6902	0.0081	0.2074	0.2650
IL-8	5 ng/ml	0.8578	0.3445	0.2565	0.5457
	25 ng/ml	0.4660	0.5290	0.8505	0.9140
	100 ng/ml	0.6332	0.1018	0.9695	0.1312
IGF-1	25 ng/ml	0.5541	0.0178	0.4733	0.3411
	100 ng/ml	0.0103	0.0286	0.5822	0.1991
	250 ng/ml	0.0001	0.0005	0.0248	0.7776
TGF- β	0.2 ng/ml	0.0016	0.0045	0.3546	0.3194
	1 ng/ml	0.0004	0.0056	0.5857	0.8621
	5 ng/ml	0.0001	0.0028	0.8121	0.1909
OSM	1 ng/ml	0.0559	0.0635	0.1045	0.1514
	10 ng/ml	0.0509	0.2374	0.6052	0.0688
	100 ng/ml	0.1248	0.6223	0.0805	0.1044
LIF	1 ng/ml	0.2045	0.1488	0.3564	0.5661
	10 ng/ml	0.2016	0.3064	0.3376	0.6072
	100 ng/ml	0.1953	0.5737	0.2241	0.6643
IL-10	1 ng/ml	0.0002	0.0638	0.5994	0.7109
	10 ng/ml	0.0012	0.0774	0.2835	0.5140

	100 ng/ml	0.0000	0.4472	0.6245	0.3201
IL-2	1 ng/ml	0.3130	0.0038	0.4374	0.8798
	10 ng/ml	0.6223	0.0857	0.7209	0.6942
	100 ng/ml	0.9471	0.5259	0.7923	0.1838
IFN- β	20 IU/ml	0.0000	0.0037	0.0000	0.0004
	100 IU/ml	0.0006	0.0021	0.0001	0.0010
	500 IU/ml	0.0003	0.0025	0.0030	0.0002
IFN- γ	20 IU/ml	0.0013	0.0046	0.0000	0.0000
	100 IU/ml	0.0001	0.0007	0.0002	0.0040
	500 IU/ml	0.0029	0.0129	0.0000	0.0010

IL-6, interleukin-6; MIP-1 α , macrophage inflammatory protein-1 α ; SDF-1 α , stromal cell-derived factor-1 α ; IL-1 β , interleukin-1 β ; IL-8, interleukin-8; IGF-1, insulin-like growth factor 1; TGF- β , transforming growth factor- β ; OSM, oncostatin-M; LIF, leukemia inhibitory factor; IL-10, interleukin-10; IL-2, interleukin-2; IFN- β , interferon- β ; IFN- γ , interferon- γ .

Supplemental Table 7. Significantly enriched pathways in CD38 high sorted cells in genome-scale CRISPR-Cas9 knockout screening

KEGG Pathway	Number of related genes	p-value
Jak-STAT signaling pathway	7	4.80E-03
Signaling pathways regulating pluripotency of stem cells	6	1.80E-02
Pancreatic cancer	4	3.40E-02

Supplemental Table 8. GO analysis in CD38 high sorted cells in genome-scale CRISPR-Cas9 knockout screening

GO term	Number of related genes	p-value
positive regulation of gene expression	10	5.60E-04
long-chain fatty acid metabolic process	4	8.10E-04
cholesterol metabolic process	5	3.10E-03
negative regulation of transcription from RNA polymerase II promoter	15	4.90E-03
cellular lipid metabolic process	4	5.70E-03
vitamin K catabolic process	2	1.80E-02
menaquinone catabolic process	2	1.80E-02
cholesterol homeostasis	4	1.90E-02
somatic stem cell population maintenance	4	2.00E-02
regulation of cell cycle	5	2.50E-02
phylloquinone catabolic process	2	2.60E-02
liver development	4	2.80E-02
cytokine-mediated signaling pathway	5	2.90E-02
transcription, DNA-templated	26	3.80E-02
positive regulation of tyrosine phosphorylation of Stat3 protein	3	4.50E-02

GO, gene ontology.